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Study on the prevalence of human papillomavirus in the anal canal of women with cervical intraepithelial neoplasia grade III

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Abstract

Objective: The objective was to evaluate the prevalence of human papillomavirus (HPV) in the anal canal of women with cervical intraepithelial neoplasia (CIN) grade III.

Study design: Two groups were compared. In group I (study group), 40 women who had undergone cervical biopsy with a histopathological result indicating CIN III were evaluated. Group II (control) consisted of 40 women with normal results from colposcopic examination and colpocytological tests. The women in group I who presented high-grade neoplasia in colpocytological tests underwent collection of material from the uterine cervix and anal canal for investigating HPV DNA using the Hybrid Capture II® technique. Colposcopy and cervical biopsy were then performed. If CIN III was confirmed, HPV DNA was investigated in the material collected. In group II, colpocytological tests and colposcopy were performed and, if normal, the procedure was similar to that followed for group I, except that no biopsy was performed.

Results: In group I, 39 women (97.5%) were positive for HPV in the uterine cervix and 14 women (35%) in the anal canal. In group II, only four women (10%) had a positive HPV test, for both the uterine cervix and the anal canal.

Conclusions: The prevalence of HPV in the anal canal of the women with CIN III was greater than in the women without CIN III.
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1. Introduction

The human papillomavirus (HPV), which is the principal cause of cervical cancer, is also associated with the development of anal cancer [1,2]. High prevalence of HPV infection in the anus has been observed among men and women who are infected by the human immunodeficiency virus (HIV) [2,3]. However, relatively little is known about the epidemiology of anal infection caused by HPV among women without HIV and its relationship with cervical infection. Although it has been established that HPV infection may be transmitted to women via anal intercourse [1,4], alternative transmission routes are possible.

A recent study showed that 27% of the women who had material collected from the anal canal and uterine cervix had HPV infection in the anal region. The findings from this study suggest that anal HPV infection is common among healthy, sexually active women and that anal and cervical HPV infection are strongly related [5].

The histopathological manifestations of HPV infection and squamous intraepithelial lesion (SIL) occur most commonly in the transformation zone of the anal and endocervical canals, which is where the columnar epithelium meets the squamous epithelium. The similarity of predisposition to HPV infection at these sites is probably a reflection of the common embryological origin of these locations [6,7].

Human papillomavirus infection can be clinical, subclinical or latent. Subclinical infection can be detected by colposcopy, while latent infection can only be diagnosed

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using molecular biology techniques, since such infection is not accompanied by clinical symptoms [8].

The prevalence of HPV in anal tissue without neoplasia has ranged from 0% [9] to 16.7% [10], while the reported prevalence of this same virus associated with anal carcinoma has been as high as 84.6% [11].

The presence of HPV in the uterine cervix has been associated with the presence of this virus in the anus. Data extrapolated from three studies have shown that similar types of HPV are found in genital, perianal and anal canal warts [12,13,14]. It also seems that HPV infection in one area of the anogenital epithelium may be followed by infection of adjacent areas by direct dissemination of the virus [15,16].

Out of more than 100 types of HPV that have been identified, approximately one-third are transmitted sexually [17] including those that cause anogenital infection and neoplasia [18,19].

The occurrence of HPV virus in the uterine cervix of women with cervical intraepithelial neoplasia (CIN) grade III is recognized to be high. Thus, it was decided to study the prevalence of these viruses in the anal canal of this group of women.

2. Materials and methods

This study was approved by the ethics committees of the Federal University of São Paulo and the Barretos Cancer Hospital. Informed consent was obtained from all participants in the study.

The study was carried out with voluntary participation by 80 women. No racial distinction was made; all the participants were at the hospital and were allocated consecutively. Two groups were formed: group I (study group) comprised women with CIN III and group II (control) comprised women with normal findings from colposcopic examinations and colpocytological tests, or with benign cell abnormalities.

Women with clinical signs or a history of vulvovaginal-perineal condyloma, immunosuppression, acquired immunodeficiency syndrome or a histopathological examination of the uterine cervix showing invasive neoplasia were excluded from the study.

The mean age in group I was 33.45 years, with a range of 18–55 years. In group II the mean age was 39.5 years, ranging from 19 to 54 years. The difference in mean age between groups I and II (lower in group I) was statistically significant ($p = 0.007$).

The mean age at coitarche in group I was 15.5 years, with a range of 10–20 years. In group II the mean age was 19.5 years, ranging from 13 to 44 years. The difference in the age when sexual activity started between groups I and II (lower in group I) was statistically significant ($p < 0.0001$).

The mean number of partners in group I was 4.5 and in group II it was 3.5. The difference in the number of partners between groups I and II (greater in group I) was statistically significant ($p = 0.0018$).

The two groups did not differ in relation to the habit of smoking ($p = 0.48$), the practice of anal intercourse ($p = 0.258$), race ($p = 0.636$) or marital status ($p = 0.059$).

The women with a diagnosis of high-grade cervical intraepithelial lesions (CIN II/CIN III) from colpocytological tests underwent video colposcopic examination. Acetic acid at a concentration of 5% was used with the purpose of identifying areas with suspected CIN III, so that biopsies could be taken.

Immediately before the video colposcopic examination or any other gynecological examination, material was collected from the uterine cervix and anal canal to investigate HPV of high and low oncogenic risk by means of the Hybrid Capture II® technique, using collectors containing the liquid medium UCM® (Universal Collection Medium; Digene, Gaithersburg, MD). The material collected for investigating HPV from the women who underwent biopsies of the uterine cervix and whose histopathological diagnosis confirmed their cases as CIN III was sent to the molecular biology laboratory.

The material for investigating HPV was collected from group I in the following manner. With the woman in the gynecological examination position, a disposable vaginal speculum was introduced and, under direct viewing of the uterine cervix, the brush was introduced into the endocervical canal to a depth of 1.0–1.5 cm and then rotated through 360° clockwise. Following this, the ectocervix was brushed and, immediately after this, the brush was inserted into the tube, inside the conserving solution.

To collect material from the anal canal, the woman was kept in the same position, gloves were changed and another brush was introduced into the anal canal, to a depth of 1.5–2.0 cm. This was then rotated five times, clockwise.

In group II, the collection of material for investigating HPV was done in the same way as described for group I, and these women also underwent video colposcopic examination and colpocytological tests.

The statistical tests were carried out using Stata 6.0® software. The significance level adopted for the tests was $p \leq 0.05$ (5%).

The Fisher test was used to compare proportions of independent populations in which, in accordance with the H0 hypothesis, at least 25% of the expected values were less than 5.

The χ^2-test was used to compare proportions of independent populations. This test was applied to analyze the following variables: smoking, anal intercourse, number of sexual partners, race and marital status.

Student's t-test was used to analyze continuous variables with normal distribution and the same variance, which was the case for the women's ages.

Welch's test was used when the variable showed different variance, as was found in relation to the coitarche.

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3. Results

The prevalence of HPV in the uterine cervix was greater in group I than in group II ($p < 0.001$; Table 1).

The prevalence of HPV in the anal canal was greater in group I than in group II ($p = 0.014$; Table 2).

The statistical analysis of the probable correlation of HPV-positive findings in the uterine cervix and anal canal in groups I and II did not show any statistically significant difference ($p = 1$; Table 3). Although the prevalence of HPV in the anal canal was greater in group I, attention was drawn to the large number of cases that were HPV-negative in the anal canal but were HPV-positive in the uterine cervix.

There was a statistically significant difference between the two groups concerning the distribution of the women in groups I and II according to their age at coitarche and number of sexual partners (Table 4).

Table 1
Occurrence of HPV in the uterine cervix in groups I and II

	Group I		Group II	
	n	%	n	%
Uterine cervix				
HPV+	39	97.5	4	10.0
HPV−	1	2.5	36	90.0
Total	40	100.0	40	100.0

HPV+ = present; HPV− = absent. Fisher's test; $p = 0.0019$.

Table 2
Occurrence of HPV in the anal canal of groups I and II

	Group I		Group II	
	n	%	n	%
Anal canal				
HPV+	14	35.0	4	10.0
HPV−	26	65.0	36	90.0
Total	40	100.0	40	100.0

HPV+ = present; HPV− = absent. Fisher's test; $p = 0.014$.

Table 3
Occurrence of HPV in the uterine cervix and anal canal

	Anal canal		Total	p
	HPV+	HPV−		
Uterine cervix				
Group I				
HPV+	14	25	39	1
HPV−	–	1	1	
Total	14	26	40	
Group II				
HPV+	–	4	4	1
HPV−	4	32	36	
Total	4	36	40	

HPV+ = present; HPV− = absent. Fisher's test; $p = 1.00$.

Table 4
Distribution of the women in groups I and II with regard to their age at coitarche and number of partners

Coitarche		Number of partners	
Age	Total (%)	n	Total (%)
Group I			
10–13	8 (20.0)	1	7 (17.5)
14–17	22 (55.0)	2 or 3	18 (45.0)
18–20	10 (25.0)	>3	15 (37.5)
>20	–		
Total	40 (100.0)		40 (100.0)
Group II			
10–13	1 (2.5)	1	21 (52.5)
14–17	13 (32.5)	2 or 3	12 (30.0)
18–20	16 (40.0)	>3	7 (17.5)
>20	10 (25.0)		
Total	40 (100.0)		40 (100.0)

$p = <0.0001, 0.0018$.

The Hybrid Capture II® method allowed the HPVs to be separated into high and low oncogenic risk types. In group I, it was found that HPV of high oncogenic risk occurred in the uterine cervix in 97.5% of the women (39 out of 40). In 85% of them (34 out of 40), only high-risk types were found. HPV of low oncogenic risk occurred in 12.5% of the women (5 out of 40) and was always found in association with high-risk HPV. In the anal canal, high-risk HPV occurred in 22.5% of the cases (9 out of 40), and in 12.5% of the cases (5 out of 40) it was in association with low-risk HPV. Low-risk HPV occurred in 25% of the cases (10 out of 40). In group II, HPV occurred in the uterine cervix in the following proportions: high-risk in 7.5% (3 out of 40) and low-risk in 2.5% (1 out of 40), and there was no association between the two types in this group. In the anal canal, these proportions were 5% (2 out of 40) for both high-risk and low-risk types, again, without any association between them.

4. Discussion

Human papillomavirus is very prevalent in certain population groups, such as HIV-positive individuals, homosexual men, and women with CIN and invasive tumors of the uterine cervix. With regard to this last group, many studies have been carried out with a focus on the prevalence of HPV in the uterine cervix, but there are few studies or reports on the occurrence of this virus in the anal canal [4,20].

Williams and Talbot [21] evaluated the prevalence of HPV in the uterine cervix and anal canal in a comparative study of two methods: dot blot (DB) and polymerase chain reaction (PCR). The prevalence found in the uterine cervix was 12% via DB and 34% via PCR, while in the anal region these figures were 22% and 67%, respectively.

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Scholefield et al. [22] used PCR and found HPV in 51% of the anal biopsies from women with CIN III and in 14% of the biopsies from the women in the control group (without CIN III).

Palefsky et al. [20] carried out a multivariate analysis of risk factors that might favor the presence of HPV in the anal canal, and found that the presence of HPV in the uterine cervix was an important independent risk factor that presented a relationship with greater prevalence of the virus in the anal canal of the women studied. Among 49 HIV-negative women from whom cervical and anal material was collected at the same time, 43% had HPV in the anal canal, whereas 24% had HPV in the uterine cervix. The diagnostic methods used were PCR and Hybrid Capture. When PCR was used, HPV was found in the anal samples of 42% of 57 HIV-negative women, and with Hybrid Capture, HPV was detected in 30% of 67 HIV-negative women.

Hernandez et al. [5] found an HPV prevalence of 27% in the anal canal and 29% in the uterine cervix. In the cases in which simultaneous collection from the uterine cervix and anal canal was carried out, HPV occurred at both sites in 13% of the patients. In that study, the women with cervical HPV infection had a risk of anal HPV infection that was three times greater.

Our study showed greater occurrence of HPV in the anal canal of the women with CIN III. The risk of anal HPV infection was 3.5 times greater in group I than in group II.

The high prevalence of HPV in the anal canal found by Scholefield et al. [22] is in agreement with the findings in our study. Our group I had greater prevalence of HPV in the anal canal than the control group, and this reinforces the idea that the group of women with CIN III would be at greater risk of anal HPV infection.

It was clearly seen that HPV was more prevalent in group I (Tables 1 and 2). However, attention was drawn to the large number of women who were HPV-negative in the anal canal but HPV-positive in the uterine cervix (Table 3). Comparative analysis of this discordance did not show any statistically significant difference. Thus, it can be inferred that factors other than the presence of HPV in the uterine cervix must be related to the presence or absence of HPV in the anal canal. In any event, the data obtained in our study cannot rule out a role for the uterine cervix as a reservoir and source of anal infection, or vice versa.

The data from group II (Table 3) corroborate the idea in the above paragraph. Despite the small number of positive cases in the uterine cervix of group II, there were no positive cases in the anal canal.

Hernandez et al. [5] found that coitarche at an early age was a risk factor for concomitant anal and cervical HPV infection. The number of sexual partners also was positively associated with concomitant anal and cervical HPV infection.

Our study showed that HPV was more prevalent in both the uterine cervix and the anal canal among the women in group I. These women had earlier coitarche, such that 75% of them started sexual activity between the ages of 10 and 17 years. On the other hand, only 35% of the women in group II had their coitarche within that age range. The number of sexual partners also differed between the two groups. In group I, 82.5% of the women had had two or more partners, while in group II, the corresponding proportion was 47.5% (Table 4).

Our study demonstrated a high prevalence of HPV in the anal canal of the women in group I (35%), in comparison with the women in group II (10%). This difference was statistically significant ($p = 0.014$).

On the basis of our findings, we believe that the women in group I were at greater risk of anal HPV infection. Nonetheless, more studies involving such women are necessary in order to corroborate our findings.

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